AMENDMENTS TO THE SPECIFICATION

Please replace the paragraph beginning at page 4, line 28 with the following:

--A preferred peptide has the structure:

H2N-Val-Phe-Arg-Ser-Leu-Lys-Tyr-Ala-Glu-Ser-Asp-COOH (SEQ. ID. NO. 13).—

Please replace the paragraph beginning at page 4, line 32 with the following:

--A fluorogenic peptide which has the following sequence:

Abz-Val-Phe-Arg-Ser-Leu-Tyr-Ala-Glu-Ser-Asp-Tyr(NO_2) (SEQ. ID. NO. 14)

has been synthesized.—

Please replace the paragraph beginning at page 8, line 12 with the following:

--The preferred peptide has the following sequence:

H2N-Val-Phe-Arg-Ser-Leu-Lys-Tyr-Ala-Glu-Ser-Asp-COOH (SEQ. ID. NO. 13).—

Please replace the paragraph beginning at page 9, line 13 with the following:

--FIG. 1 shows the comparative protein sequences of SKI-1 deduced from rat, mouse and human cDNAs (SEQ. ID. Nos. 2, 4, and 6 encoded by nucleic acids SEQ. ID. Nos: 1, 3 and 5 respectively). The position of the predicted end of the 17aa signal peptide is shown by an arrow. The active sites Asp 218, His249 and Ser414, as well as the oxyanion hole Asn 338 are in bold, shaded and underlined characters. The positions of the 6 potential N-glycosylation sites are emphasized in bold. The conserved shaded **CL**DDSHRQK**DC**F**W** (SEQ. ID. NO. 77) sequence fits in the consensus signature for growth factors and cytokine receptors family. Each of the two boxed sequences was absent in a number of rat clones. The predicted transmembrane segment is in bold and underlined--

Please replace the paragraph beginning at page 14, line 16 with the following:

--FIG. 21 illustrates the purification and identification of secreted recombinant pro-SKI-1. [A] Media obtained from HK293 cells stably expressing FL-SKI-1 were concentrated and sequentially applied to C4 semi-preparative column (not shown) followed by a C4 analytical RP-HPLC columns, and then eluted by the indicated linear CH3CN gradient. [B] The fractions labeled I-IV were collected and analyzed by Western blotting using the primary antiserum Ab:P. [C,D] Proteins contained in fraction IV were separated on a 10% SDS-

PAGE reducing gel. Following electrotransfer, the proteins were stained with Ponceau Red. The immunoreactive 14kDa and non-immunoreactive but colored ~ 4.5 kDa [D] polypeptides were excised and submitted to N-terminal sequencing (X represents an undefined residue). [E] Mass spectrometric analysis by MALDI-TOF spectrometry of fraction IV. The C-terminal residues sites believed to corresponding to the three ~14 kDa polypeptides are underlined, whereas the expected (potential) cleavage sites are indicated by dashed arrows (SEQ. ID. NO. 108).

Please replace the paragraph beginning at page 16, line 23 with the following:

-- Polymerase Chain Reaction and Sequencing. Most reverse transcriptase polymerase chain reactions (RT-PCR) were performed using a Titan One Tube RT-PCR system (Boehringer Mannheim) on 1 μg of total RNA isolated from either a human neuronal cell line (IMR-32), mouse corticotrophic cells (AtT20), or rat adrenal glands using a TRIzol reagent kit (Life Technologies). The active site degenerate primers were: His (sense) 5' GICA(C,T)GGIACI(C,T)(A,T)(C,T)(G,T)(T,G)IGCIGG-3'(SEQ. ID. NO. 15) and Ser (antisense) 5'-CCIG(C,T)IACI(T,A)(G,C)IGGI(G,C)(T,A)IGCIACI(G,C)(A,T)GTICC-3'(SEQ. ID. NO. 16) based on the sequences GHGT(H,F)(V,C)AG (SEQ. ID. NO. 17) and GTS(V,M)A(T,S)P(H,V)V(A,T)G (SEQ. ID. NO. 18) respectively. The amplified 525 bp products were sequenced on an ALF DNA sequencer (Pharmacia). To obtain the full length of rat and mouse SKI-1, we used PCR primers based on the human (12) and mouse sequences, in addition to 5' (13) and 3' (14) RACE amplifications. To avoid errors, at least three clones of the amplified cDNAs were fully sequenced. The GenBank accession numbers of the 3788 bp mouse mSKI-1 cDNA and 3895 bp rat rSKI-1 are AF094820 and AF094821, respectively.--

Please replace the paragraph beginning at page 19, line 2 with the following:

--Protein Sequence Analysis of SKI-1. We first aligned the protein sequences within the catalytic domain of PC7 (21), yeast subtilases and bacterial subtilisins together with that of a novel subtilisin-like enzyme from Plasmodium falciparum(J-C. Barale et al., submitted) . This led to the following choice of conserved amino acids around the active sites His and Ser: GHGT(H/F)(V/C)AG (SEQ. ID. NO. 17) and GTS(M/V)A(T/S)P(H/V)V(A/T)G(SEQ. ID. NO. 18) respectively. Thus, using degenerate oligonucleotides coding for the sense His and antisense Ser consensus sequences we initiated a series of RT-PCR reactions on total RNA (see Materials and Methods) and isolated a 525 bpcDNA fragment from the human neuronal cell line IMR-32.

This sequence was found to be 100% identical to that reported for a human cDNA called KIAA0091 (Accession No. D42053) obtained from a myeloid KG-1 cell line (12) and 88 % identical to that of a 324 bp EST sequence (Accession No. H31838) from rat PC12 cells. We next completed the rat and mouse cDNA sequences following RT-PCR amplifications of total RNA isolated from rat adrenal glands and PC12 cells, and from mouse AtT20 cells. Starting from the equivalent rat and mouse 525 bp fragments, the complete sequences were determined using a series of RT-PCR reactions with human-based oligonucleotides in addition to 5' (13) and 3' (14) RACE protocols. As shown in Fig. 1, alignment of the protein sequence deduced from the cDNAs of rat, mouse and human SKI-1 revealed a high degree of conservation. Rat and mouse SKI-1 share 98% sequence identity and a 96% identity to human SKI-1. Interestingly, within the catalytic domain (Asp^{218} to Ser^{414}) the sequence similarity between the three species is 100%. Analysis of the predicted amino acid sequence suggests a 17 aa signal peptide, followed by a putative pro-segment beginning at Lys^{18} and extending for some 160-180 amino acids. The proposed catalytic domain encompasses the typical active sites Asp^{218}, His^{249} and Ser^{414} and the oxyanion hole Asn^{338}. This domain is followed by an extended C-terminal sequence characterized by the presence of a conserved growth factor/cytokine receptor family motif C^{849}LDDSHRQKDCFW^{861} (SEQ. ID. NO. 77). This sequence is then followed by a potential 24 aa hydrophobic transmembrane segment and a less conserved 31 aa cytosolic tail that remarkably consists of 35% basic residues. Some of the clones isolated from rat adrenal glands suggested the existence of alternatively spliced rSKI-1 mRNAs in which the segments coding for aa 430-483 or 858-901 are absent. Finally, the phylogenetic tree derived from the alignment of the catalytic domain of SKI-1 with subtilases (22) suggests that it is an ancestral protein that is closer to plant and bacterial subtilases than to either yeast or mammalian homologues (not shown).—

Please replace the paragraph beginning at page 22, line 27 with:

--In FIG. 6B, we present the N-terminal microsequence analysis of [^{35}S]Met-labeled 32 32 kDa proBDNF and [^{3}H] Leu-labeled 28 kDa BDNF. The sequence of the 32 kDa form revealed the presence of an [^{35}S] Met at position 3 (Fig. 6B), which is in agreement with the proposed sequence of human proBDNF (27) resulting from the removal of an 18 aa signal peptide cleaved at GMCLA18↓APMK (SEQ. ID. NO. 78) site. The N-terminal sequence of the 28 kDa product revealed a [^{3}H] Leu at positions 2, 13 and 14 (Fig. 6B). This result demonstrates the 28 kDa BDNF is generated by a unique cleavage at Thr^{57} in the sequence: $RGLT^{57}$ ↓SLADTFEHVIEELL (27) (SEQ. ID. NO:.79).--

Please replace the paragraph beginning at page 24, line 26 with:

--This work provides the first evidence for the existence of a mammalian secretory Ca^{2+}-dependent serine proteinase of the subtilisin-kexin type that selectively cleaves at non-basic residues. Thus, SKI-1 processes the 32 kDa human proBDNF at ~~an~~ [a] KAGSRGLT↓SL (SEQ. ID. NO. 80) sequence generating a 28 kDa form, which may have its own biological activity (Mowla, S.J. *et al., submitted*). Such a cleavage site is close to the consensus site deduced from a large body of work. Done with the PCs, whereby and (R/K)-$(X)_n$ -R↓X-(L/I/V), [where n=0, 2, 4 or 6] motif is favored by most Pcs (1-3, 28). Note that in the SKI-1 site, P1 Arg is replaced by Thr and an aliphatic Leu is present at P2', an amino acid also favored by PCs (1-3, 28). Several proteins are known to be cleaved following Thr. These include human anti-angiogenic platelet factor 4 (6; QCLCVKTT↓SQ (SEQ. ID. NO. 81) and angiostatin (7; KGPWCFTT↓DP (SEQ. ID. NO. 82)), the neuroendocrine α-endorphin (4; KSQTPLVT↓LF (SEQ. ID. NO. 83)), the ADAM-10 metalloprotease (8; LLRKKRTT↓SA (SEQ. ID. NO. 84)), as well as the amyloidogenic peptide Aβ43 (10; VGGVVIAT↓VI (SEQ. ID. NO. 85)).—

Please replace the paragraph beginning at page 25, line 6 with:

--Interestingly, comparison of the phylogenetically highly conserved sequence of proBDNF revealed an insertion of hydroxylated amino acids (Thr and Ser) just after the identified SKI-1 cleavage site of human proBDNF. Thus, in rat and mouse proBDNF, two threonines are inserted (RGLTTT-SL (SEQ. ID. NO. 86)) and in porcine proBDNF five serines added (RGLTSSSSS-SL (SEQ. ID. NO. 87))(27). These observations raised a number of questions: (i) do these insertions affect the kinetics of proBDNF cleavage by SKI-1? (ii) does SKI-1 recognize both single and pairs of Thr and Ser and combinations thereof? (iii) is the presence of a basic residue at P4, P6 or P8 important for cleavage? and (iv) similar to enzymes cleaving at basic residues (29), does the possible phosphorylation at specific Thr or Ser residues affect substrate cleavability by SKI-1? Answers to these questions are provided hereinbelow.—

Please replace the paragraph beginning at page 25, line 17 with:

--Biosynthetic analysis of the zymogen processing of proSKI-1 demonstrated a two-step ER-associated removal of the pro-segment (Fig. 4). Furthermore, analysis of the [$^{35}SO_4$]-labeled SKI-1 demonstrated only the presence of sulfated 106 and 98 kDa forms but not that of either the 148 or 120 kDa forms recognized by the Pro-segment antiserum (*not shown*). Since sulfation occurs in the *trans* Golgi network, this confirms that the removal of the pro-segment occurs in the ER. Like furin and PC5-B (1-3, 24) the membrane bound 106 kDa SKI-1 is transformed into a soluble

98 kDa form that is released into the medium by an as yet unknown mechanism. The secreted 98 kDa SKI-1s is enzymatically active since it processes proBDNF *in vitro* (Fig. 7). Numerous attempts to sequence the SDS-PAGE purified [^{3}H]Leu and Val-labeled 148 kDa and 98 kDa forms, resulted in ambiguous results, suggesting that SKI-1 is refractory to N-terminal Edman degradation. Presently, we cannot define the two zymogen cleavage sites leading to the sequential formation of the 120 kDa proSKI-1b and 106 kDa SKI-1 deduced by pulse (Fig. 4) and pulse-chase studies (*not shown*). Examination of the pro-segment sequence (Fig. 1), the species-specific proBDNF motif potentially recognized by SKI-1 (*see above*), and the alignment of SKI-1 with other subtilases (22), suggests two possible conserved sites: RNNPSS^{95}↓DYPS (SEQ. ID. NO. 88) and RHSS^{182}↓RRLL (SEQ. ID. NO. 89). Both sites predict a cleavage after pairs of Ser with either a P6 or a P4 Arg, respectively.--

Please replace the paragraph beginning at page 28, line 4 with:

--To study the effect of the SKI-1 prosegment (ProSki-1) on the SREBP processing and mediated transcriptional activity we isolated a cDNA fragment covering the 188 amino acids that make up the signal peptide and the prosegment of SKI-1 including the predicted cleavage site RRLL^{176} (SEQ. ID. NO. 90). This autocatalytic cleavage site was confirmed by mass spectral analysis and amino acid sequencing by other investigators [19]. We isolated stable cell lines overexpressing SREBP-1 (neo resistance) and ProSki-1 plasmid (zeo resistance). A background SREBP-1 overexpression was used in order to improve detection of nuclear NH_2-terminal segment of SREBP in immunoblot experiments.—

Please replace the paragraph beginning at page 30, line 25 with:

—Plasmid constructions: SKI-1 prosegment containing aa 1-188 was isolated by PCR using following oligonucleotides: [5' GGA TCC GAA GAA ACA TCT GGG CGA CAGA 3' (SEQ. ID. NO. 19)] and [5' CTC GAG GGC TCT CAG CCG TGT GCT 3'(SEQ. ID. NO. 20)] and cloned into PCR 2.1 TA cloning vector for sequencing. After that it was subcloned into the $pcDNA_{3zeocin}$ vector (Invitrogen) (BamHI / HindIII sites) for transfections.—

Please replace the paragraph beginning at page 31, line 7, with:

--Northern blotting: 20 μg of total RNA was electroforetically separated in an 1.0 % agarose gel, and transferred to Hybond N$^+$ filters (Amersham, city, state) by capillary blotting. After transfer filters were crosslinked by UV irradiation in a Stratalinker (Stratagene). Filters were

prehydridized at 42 °C for 1 hour and hybridized with random labeled ^{32}P cDNA probes for 16-20 hours. Ultrahyb buffer (Ambion) was used. After hybridization filters were washed and exposed to film for indicated time and bands were quantified by densitometry. ~~Following primer~~ Primer pairs were used to clone cDNA probes: HMG CoA reductase [5' GAG GAA GAG ACA GGG ATA AAC 3' (SEQ. ID. NO. 21)], [5' GGG ATA TGC TTA GCA TTG AC 3' (SEQ. ID. NO. 22)], farnesyl diphosphate [5' AGC CCT ATT ACC TGA ACC TG 3' (SEQ. ID. NO. 23)] , [5' GAA TCT GAA AGA ACT CCC CC 3' (SEQ. ID. NO. 24)], Fatty acid synthase [5' TTC CGA GAT TCC ATC CTA CG 3' (SEQ. ID. NO. 25)], 5' TGC AGC TCA GCA GGT CTA TG 3'(SEQ. ID. NO. 26)], Acetyl CoA carboxylase [5' TCT CCT CCA ACC TCA ACC AC 3'(SEQ. ID. NO. 27)], [5' CCA GCC TGT CAT CCT CAA TAT C 3' (SEQ. ID. NO. 28)], SREBP-1 [5' GGA GCC ATG GAT TGC ACT TTC 3' (SEQ. ID. NO. 29)], [5' AGG AGC TCA ATG TGG CAG GA 3' (SEQ. ID. NO. 30)], LDL-receptor ~~[5' 3'], [5' 3']~~ (Data not shown). Amplification products were cloned into pGEM (Promega) and sequenced. 18S cDNA was purchased from Ambion.—

Please replace the paragraph beginning at page 32, line 5, with:

--*Vaccinia Virus Recombinant of BTMD-SKI-1* - The preparation of a soluble form of hSKI-1 involved the initial amplification by polymerase chain reaction (PCR) of a 1250 base pair (bp) product encompassing nucleotides (nts) 491-1740 of the hSKI-1 cDNA (12), which includes the initiator methionine. The sense (s) and antisense (as) oligonucleotides were 5' GTGACCATG-AAGCTTGTCAACATCTGG 3' (SEQ. ID. NO. 31) and 5' ACACTGGTCCCTGAGAGGGCCCGGCA 3' (SEQ. ID. NO. 32) respectively. This completely sequenced fragment, which had been inserted into the PCR2.1 TA cloning vector (Invitrogen), was first digested with NotI and AccI. It was then ligated with the similarly digested full-length hSKI-1 cDNA 3.5 kb product, resulting in a product called 5' hSKI-1-FL. In order to obtain a soluble form of hSKI-1 with a hexa-His sequence just before the stop codon, PCR amplification was carried out using the sense and antisense oligonucleotides:

5' ATTGACCTGGACAAGGTGGTG3' (SEQ. ID. NO. 33) and

5'GGATCCTCTAGATCAGTGGTGGTGGTGG-TGGTGGTGCTCCTGGTTGTAGCGGCCAGG 3' (SEQ. ID. NO. 34). This resulted in a 165 bp fragment encoding the C-terminal sequence PGRYNQE^{997}-(H_6)* (SEQ. ID. NO. 91) (10). Following digestion with 5' EcoNI and 3' XbaI, the product was ligated to the aforementioned

and similarly digested 5' hSKI-1-FL. This cDNA, coding for BTMD-SKI-1 ending with a hexa-His sequence, was then transferred to the BamH1/XbaI site of the (VV) transfer vector PMJ601. A recombinant was then isolated as previously reported (13). The VV recombinant of full-length hSKI-1 has been described (10).

Please replace the paragraph beginning at page 33, line 1, with :

--*Isolation and Purification of Recombinant hSKI-1 Prosegments* - Three N-terminal fragments of hSKI-1 were isolated by PCR using a common (s) oligonucleotide [5' GGATCCGAAGAAACATCTGGGCGACAGA 3' (SEQ. ID. NO. 19)] and one of three (as) oligonucleotides [5' CTCGAGGGAGAGGCTGGCTCTTCG 3' (SEQ. ID. NO. 35)], [5' CTCGAGGGCTCTCAGCCGTGTGCT 3' 3' (SEQ. ID. NO. 20)], or [5' CTCGAGTGTCTGGGCAACCTGGCGCGGG 3' (SEQ. ID. NO. 36)]. These prosegment fragments, ending at aa 169, 188, and 196 (10), were cloned in the PCR 2.1 TA cloning vector for sequencing. Then they were transferred into the BamHI / XhoI sites of the bacterial expression vector pET 24b (Novagen). These recombinants were transformed into the *E. Coli* strain BL21. Protein expression was induced with 1mM isopropyl β-D-thiogalactoside and the cultures were grown for 3h at 37°C. The cell pellets were sonicated on ice in a binding buffer containing 6M guanidine-HCl (Novagen) until a clear solution was obtained. The clarified and filtered solution was then applied to a nickel affinity column (Novagen) and eluted with 500 mM imidazole. The eluates were dialyzed overnight at 4°C against 50 mM sodium acetate (pH 7). The protein precipitate was solubilized with glacial acetic acid, filtered through a 0.45 μm disk and further purified on a 5 μm C4 column (0.94 x 25 cm; Chromatographic Sciences Company Inc; CSC) by reverse-phase high performance liquid chromatography (RP-HPLC). The purity was assessed by Coomassie staining and the identity of the products verified by mass spectrometry on a Matrix Assisted Laser Desorption Time of Flight (MALDI-TOF) Voyageur DE-Pro instrument (PE PerSeptive Biosystems). The amounts of prosegments were determined by quantitative amino acid analysis (13).--

Please replace the paragraph beginning at page 34, line 18, with :

--*Synthesis of Peptide Substrates* - All Fmoc amino acid derivatives (L-form), the coupling reagents, and the solvents for peptide synthesis were purchased from PE Biosystems Inc. (Framingham, Mass, USA), Calbiochem (San Diego, Ca, USA), or Richelieu Biotechnologies

(Montréal, QC, Canada). The various linear synthetic peptides and internally quenched fluorogenic (Q-) substrates reported in this article are: (I) **hproBDNF(50-63)**: KAGSRGLTSLADTF (SEQ. ID. NO. 37), (II) **hSREBP-2(504-530)**: GGAHDSDQHPHSGSGRSVLSFESGSGG (SEQ. ID. NO. 38), III) **hSKI-1(174-191)**: WHATGRHSSRRLLRAIPR (SEQ. ID. NO. 39), (IV) **hSKI-1(174-188+LE)**: WHATGRHSSRRLLRALE (SEQ. ID. NO. 40), (V) **hSKI-1(182-188+LE)**: SRRLLRALE (SEQ. ID. NO: 41), (VI) **hSKI-1(156-172)**:WQSSRPLRRASLSLGSG (SEQ. ID. NO. 42), (VII) **hSKI-1(187-201)**: RAIPRQVAQTLQADV (SEQ. ID. NO. 43), (VIII) **hSKI-1(128-136)**: PQRKVFRSL (SEQ. ID. NO. 44), (IX) **hSKI-1(128-142)**: PQRKVFRSLKYAESD (SEQ. ID. NO. 45), (X) **Q-hSKI-1(132-142)**: Abz-VFRSLKYAESD-Y(NO_2)-A (SEQ. ID. NO. 46), (XI) **Q-hSKI-1(134-142)**: Abz-RSLKYAESD-Y(NO_2)-A (SEQ. ID. NO. 47). Except for the first two peptides, which were purchased from the Sheldon Biotechnology Institute (McGill University, QC, Canada), all other peptides were synthesized with the carboxy-terminus in the amide form. Peptides III-XI were prepared on a solid phase peptide synthesizer (Pioneer model, PE Biosystems) using either 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluophosphate (HBTU) / N-hydroxybenzotriazole (HOBT) or HATU (O-[7-azabenzotriazol-1-yl]-N,N,N',N'-tetramethyluronium hexafluorophosphate) / diisopropyl ethyl amine (DIEA)-mediated Fmoc chemistry with PAL-PEG unloaded resin and the standard side chain protecting groups (16). For the incorporation of the two unnatural amino acids [Abz and Y(NO_2)], an extended coupling cycle was used instead of either the standard or fast cycles.--

Please replace the paragraph bridging at pages 37 and 38, which starts with "To further characterize the prosegment [...]" with :

--To further characterize the prosegment of SKI-1, we took advantage of a stable transfectant of FL-SKI-1 in human HK293 cells that we had made previously (10). This system has the added advantage that the possibility of VV overexpression artifacts influencing the processing of the prosegment is eliminated. Concentrated culture medium from these cells (serum-free) was purified via RP-HPLC using first a semi-preparative C4 column (*not shown*) followed by an analytical C4 column (Fig. 21A). The eluted fractions were analyzed by Western blot using Ab:*P* (Fig. 21B). Immunoreactive peptides ranging from ~4.5-24 kDa were apparent. N-terminal sequencing of the very abundant ~14 kDa protein in fraction IV (Fig. 21C) revealed a major sequence starting at ~~Gly~~^{18} Gly^{17} of pre-proSKI-1 (10,12). This clearly defines the signal peptidase cleavage site as ~~LWLLC~~^{17} LWLLC^{16} ↓GKKHLG (SEQ. ID. NO. 92), which is one aa before that

predicted by signal peptidase cleavage site algorithms (10,11). The N-terminal sequence of the ~4.5 kDa polypeptide (Fig. 21D) revealed that it starts at Pro^{143}, indicating a cleavage at the sequence $KYAESD^{142}\downarrow$PTVPCNETRWSQK (SEQ. ID. NO. 93). This fragment is most likely the product of cleavage between Asp and Pro that may be caused by the acidic conditions encountered in either RP-HPLC, Edman sequencing (20), or sample preparation for SDS-PAGE analysis (21). An unexpected benefit of this cleavage was our finding that phenylthiohydantoin (PTH)-Asn^{148}, which occurs in the putative N-glycosylation site AsnGluThr was readily detected in this sequence. Thus, the predicted N-glycosylation site Asn^{148} within the prosegment of SKI-1 is not employed, at least in this expression system. This conclusion was also supported by the prosegment's resistance to endo H and endo F digestion (*not shown*). Of the two eukaryotic subtilases known to contain a potential N-glycosylation AsnGluThr site, *i.e.* kexin (22) and SKI-1 (10), it appears that at least the latter's prosegment is not N- glycosylated. Finally, the separation of the above prosegment fragments from mature SKI-1 using RP-HPLC (Fig. 21A,B) and non-reducing SDS-PAGE (*not shown*), suggests that none of the Cys residues in the prosegment (10) are linked by disulfide bridges to the rest of the enzyme.--

Please replace the paragraph bridging at pages 38 and 39, which starts with "As a preliminary means [...]" with :

-- As a preliminary means of characterizing the SKI-1 prosegment fragments, MALDI-TOF analysis (Fig. 21E) of fraction IV from Fig. 21B was carried out. Three major molecular ions of masses 13,351, 13,518, and 13,685 Da were detected, with an expected error of ± 25 Da for this mass range. Combined with the previous N-terminal sequencing results of the ~14 kDa peptide (Fig. 21C), these mass values indicate that this peptide has heterogeneous C-termini that are derived from cleavages near the sequence **RK**V**FRSLK**^{137}(SEQ. ID. NO. 94), as indicated in Fig. 21E. In fact this region contains three potential SKI-1 cleavage sites (8) with an R or K at the P4 position and either an F, R or K at the P1 position. Although the calculated molecular masses of 13,339, 13,496 and 13,696 for the polypeptides G^{17}KK---**RK**VF^{133} (SEQ. ID. NO. 95), G^{17}KK---**RK**V**FR**^{134}(SEQ. ID. NO. 96) and G^{17}KK---**RK**V**FRSL**^{136} (SEQ. ID. NO. 97) espectively, match within experimental error (± 22 Da) the observed masses in Fig. 21E, these assignments should only be taken as a first indication (*see below*). Moreover, the predicted G^{17}KK---**RK**V**FRSL**^{136} (SEQ. ID. NO. 98) Lys^{137} followed by basic carboxypeptidase cleavage of the C-terminal Lys (23). Since we were unable to obtain consistent mass spectra of the ~4.5 kDa polypeptide that was sequenced in Fig. 21D, we could not use this technique to approximate its C-terminus, which

presumably corresponds to the C-terminus of the processed SKI-1 pro-segment. We therefore resorted to synthetic peptide cleavage as a tool to accurately define potential prosegment cleavage sites.--

Please replace the paragraph beginning at page 39, line 12, with :

Analysis of Synthetic Prosegment-derived Peptide Cleavages-Based on our detection of ~26[ ]and 24 kDa SKI-1 prosegment products (Fig. 20), as well as on a mutagenesis study of SREBP-2 cleavage sites (8), we synthesized three SKI-1 prosegment peptides encompassing potential, C-terminal, autocatalytic cleavage sites (10,11). All contain Arg at P4 and either Leu, Lys, Ala or Phe at P1 (peptides III, VI and VII shown in Table II-A). Of these peptides containing only native sequences, the only one with detectable cleavage by SKI-1-containing concentrated medium (from either VV:BTMD-SKI-1-infected BSC40 cells or SKI-1 transfected HK293 cells) was peptide III (WHATGRHSS**R**RL**L**$^{186\downarrow}$RAIPR (SEQ. ID. NO. 39) (see Table II-A). No cleavages were observed when VV:WT-infected or empty vector-transfected media were used (*not shown*). Metal chelation chromatography-purified enzyme further supported that this cleavage is effected by SKI-1 (Fig. 22A; peptide III), and the products were positively identified via mass spectrometry.--

Please replace the paragraph bridging at pages 39 and 40, which starts with "Similarly, based on the mass [...]" with:

--Similarly, based on the mass spectrometry data in Fig. 21E, we synthesized two peptides (VIII and IX) encompassing the putative internal processing site(s) of the SKI-1 prosegment. Both were cleaved at multiple locations by SKI-1-containing concentrated medium from HK293 transfectants (*not shown*). Further analysis revealed that one of these cleavages, corresponding to PQ**R**KVF$^{133}\downarrow$**R**SL (SEQ. ID. NO. 44), was as prevalent in empty vector-transfected HK293 medium as in SKI-1-transfected medium (see. Table III-A, peptide VIII). In contrast, the PQ**R**KVF**R**SL**K**$^{137}\downarrow$ YAESD (SEQ. ID. NO. 45) cleavage was only seen in SKI-1-containing medium. This cleavage was also confirmed using metal chelation chromatography-purified enzyme (Fig. 22B; peptide IX) and mass spectrometry to identify the products. However, also clearly visible are the PQ**R**KVF$^{133}\downarrow$**R**SL**K**YAESD (SEQ. ID. NO. 45) cleavage products. We acknowledge that there could be residual contaminating proteases in our purified SKI-1 preparations (minor bands were visible on colloidal gold-stained membranes of SKI-1 preparations). Thus, while we are confident that SKI-1 cleaves its prosegment at the C-terminal

WHATGRHSS**R**RL**L**^{186}↓RAIPR (SEQ. ID. NO. 39) site and at the internal PQ**R**KVF**R**SL**K**^{137}↓ YAESD (SEQ. ID. NO. 45) site, our data do not allow us to rule out SKI-1-mediated cleavage at the PQ**R**KVF^{133}↓**R**SL**K**YAESD (SEQ. ID. NO. 45) site.--

Please replace the paragraph bridging at pages 40 and 41, which starts with "*In Vitro Kinetic Properties* [...]" with:

--*In Vitro Kinetic Properties of SKI-1: Comparative Analysis of Synthetic Peptide Cleavages* -_In a previous report (10), sSKI-1 was shown, to cleave the 32 kDa proBDNF into a 28 kDa form at the **R**GL**T**↓SL (SEQ. ID. NO. 99) sequence *in vitro* with a pH optimum close to neutrality. Similar to PCs (1-3), we suggested that SKI-1 might be a Ca^{2+}-dependent enzyme since the calcium ionophore A23187 inhibited the *ex vivo* cleavage of proBDNF (10). In order obtain kinetic analyses of defined SKI-1 substrates, we examined a 14 aa peptide spanning the hproBDNF processing site (10), K^{50}AGS**R**GL**T**↓SLADTF^{63} (SEQ. ID. NO. 37) peptide I) and a 27 aa hSREBP-2-related peptide (8), G^{504}GAHDSDQHPHSGSG**R**SV**L**↓SFESGSGG^{530} peptide II). Concentrated SKI-1-containing medium (from either VV:BTMD-SKI-1-infected BSC40 cells or SKI-1 transfected HK293 cells) was reacted with these peptides at pH 6.5, followed by MALDI-TOF mass spectrometric analysis of the RP-HPLC-purified products. The expected cleavages were confirmed and did not occur using WT-/empty vector-derived media (Fig. 23). Again, the metal chelation chromatography-purified enzyme generated the same products as the concentrated media (not shown). We then demonstrated that the optimal pH and calcium concentrations for efficient cleavage of the hSREBP-2 peptide (II) are pH 6.5 and 2 mM Ca^{2+}, respectively (Fig. ~~24~~ 23). Interestingly, the pH optimum observed with the the proBDNF peptide (I) is sharper than that obtained with peptide II. In the former case, the enzyme still retains about 30% of its activity at pH 5.0 and 55 % of its activity at pH 8.5 (Fig. 24A). Similar results for the pH optimum of peptide II cleavage were obtained with metal chelation-purified BTMD-SKI-1 (*not shown*). In contrast, however, the pH optimum of peptide IX with the purified enzyme was 8.0, with no activity detectable below pH 5.5.--

Please replace the paragraph bridging at pages 41 and 42, which starts with "In order to develop [...]" with:

--In order to develop a convenient *in vitro* assay for SKI-1, we designed a number of internally quenched fluorogenic substrates and tested their cleavage efficacy by SKI-1. The two best peptides encompassed the processing site RSLK↓ within the hSKI-1 prosegment (peptides X and

XI, Table II-A). Mass spectrometric analysis confirmed that both peptides were cleaved at the RSLK↓ (SEQ. ID. NO. 100) site by shed SKI-1 derived from HK293 cell transfects, but not by medium obtained from HK293 empty vector transfectants. This processing generated the fluorescent N-terminal peptides Abz-VFRSLK (SEQ. ID. NO. 101), or Abz-RSLK (SEQ. ID. NO. 102), and a non-fluorescent C-terminal peptide YAESDY(NO_2)-A (SEQ. ID. NO. 103) *not shown*). Measurements of kinetic parameters demonstrated that peptides X and XI are about 3- and 16-fold better substrates than the C-terminal prosegment peptide IV (Tables I-B and III), suggesting that the shorter peptide XI may be the best SKI-1 substrate tested to date. This cleavage was completely abolished in the presence of 10 mM EDTA, in agreement with the Ca^{2+}-dependence of SKI-1 activity (Fig. 24B).--

Please replace the paragraph beginning at page 42, line 8, with:

--*SKI-1 Inhibition by its Prosegment* -_One important question remaining is whether the SKI-1 prosegment functions as an inhibitor of its enzymatic activity, analogous to the prosegments of other subtilases (3). We thus prepared prosegment constructs, designated ending near the proposed C-terminal processing site $RRLL^{186}$ (SEQ. ID. NO. 90) (Fig. 22A): PS1, extending to Leu^{169}; PS2, extending to Ala^{188}; and PS3, extending to Leu^{197} To each C-terminus we coupled a hexa-His tag. These prosegment constructs were expressed in bacteria and purified by Ni^{2+}-chelation chromatography followed by RP-HPLC (*see Experimental Procedures*). The purity of these prosegments was confirmed by SDS-PAGE/Coomassie staining and aa analysis (*not shown*). A summary of the inhibitory potency of each prosegment using peptide IV as a substrate is shown in Table V. Kinetic analysis using Dixon plots (15) indicated a competitive inhibition mechanism (*not shown*). Although PS2 exhibits the best apparent inhibitory constant ($K_{i(app)}$ = 97 nM), PS3 ($K_{i(app)}$ = 127 nM) and PS1($K_{i(app)}$ = 182 nM) are similarly potent SKI-1inhibitors. When PS2 was digested with carboxypeptidase B to eliminate the His-tag, its inhibitory potency was not affected (*not shown*), confirming that this tag is not responsible for the observed inhibition. We also tested the inhibitory activity of the RP-HPLC-fractionated native prosegment (see Fig. 21). Only, the material from fraction IV, which included the full-length ~24 kDa prosegment, was inhibitory, whereas that of the others , including the ~14 kDa peptide alone or in combination with smaller fragments, were not inhibitory (*not shown*).--

Please replace the paragraph beginning at page 43, line 4, with:

--Many peptidyl hydrolases, including subtilases, possess a prodomain which acts both as an intramolecular chaperone and a highly potent inhibitor of its associated protease (24,25). Activation of the enzyme typically requires release of the prosegment in an organelle-specific manner. For furin (26) the release occurs in the TGN, whereas for PC1 and PC2 (27) it occurs in immature secretory granules. The data presented in this report demonstrate that SKI-1 is unique among the mammalian subtilases, since both the C-terminal and internal cleavages of its prosegment occur in the ER. Hence, this enzyme does not appear to require an acidic environment for activation, assuming, by analogy with other subtilases (3), that prosegment release is the crucial step leading to zymogen activation. We propose the following sequence of events presumably leading to SKI-1 activation: **1)** The signal peptide is removed in the ER by a signal peptidase cleavage at LVVLLC^{17}↓GKKHLG (SEQ. ID. NO. 92) Fig. 21C). **2)** The prosegment is processed into a non-N-glycosylated polypeptide with an apparent molecular mass of ~24-26 kDa (Fig. 20). **3)** This prosegment is further processed into 14, 10 and 8 kDa intermediates (Fig. 20). While these multiple cleavages may be catalyzed by SKI-1 itself, the participation of other proteases cannot be excluded. The major cleavages leading to the formation of the ~24 and ~14 kDa products occur within 10 min, and the other secondary ones within 30 min (*not shown*). Since treatment of cells with BFA did not significantly alter these processing events, they most likely occur in the ER (Fig. 20). It is possible that the generation of prosegment fragments from the ~24-26 kDa pro-form leads to a loss of inhibition in a fashion similar to that of subtilisin E (24,25). Indeed, our results demonstrate that while the full-length prosegment is inhibitory, its ~14 kDa product is not. Surprisingly, some pro-region-derived polypeptides are found associated with SKI-1 in cell culture media. Thus, in contrast to furin (26), the low pH and high Ca^{2+} concentrations prevailing in the TGN do not lead to propeptide dissociation. High ionic concentrations (up to 1M NaCl) such as those used in immunoprecipitation (Fig. 19B) and metal chelation protein purification (Fig. 19C) also do not disrupt the complex. It is only during RP-HPLC purification (Fig. 21A), in the presence of strong acids and organic solvents, that the prosegment peptides dissociate from SKI-1. These data suggest that hydrophobic interactions may be critical, as is the case for subtilisin (24,25).--

Please replace the paragraph beginning at page 44, line 1, with:

--To distinguish the SKI-1 prosegment autoprocessing sites (C-terminal and internal) from several closely situated candidate sites, we employed a combination of mass spectrometry and synthetic peptide digestion. In the case of the C-terminal site, only one of three candidate peptides (III) was processed by SKI-1 (Table II-A), indicating that **R**RL**L**^{186}↓RAIP (SEQ. ID. NO. 104) is the most likely autoprocessing site. For the internal site, preliminary mass spectrometric data suggested three distinct cleavages occuring within the sequence PQ**RK**VF**R**SL**K**YAESD^{142} (SEQ. ID. NO. 45) (Fig. 21E). Two of the three possible sites (PQ**RK**VF^{133}↓**R**SL**K**YAESD (SEQ. ID. NO. 45) and PQ**RK**VF**R**^{134}↓SL**K**YAESD (SEQ. ID. NO. 45) appeared to satisfy the proposed SKI-1 recognition motif requiring a P4 basic residue (8). The third possibility (PQ**RK**VF**R**SL^{136}↓**K**YAESD (SEQ. ID. NO. 45) could be considered by assuming the cleavage actually occurred at PQ**RK**VF**R**SL**K**^{137}↓YAESD (SEQ. ID. NO. 45) followed by endogenous, basic carboxypeptidase removal of the C-terminal Lys residue (23). Assays carried out *in vitro* with synthetic peptides corresponding to this region of the prosegment (peptides VIII and IX) produced the same cleavage products (*not shown*), but only the PQ**RK**VF**R**SL**K**^{137}↓YAESD cleavage was unique to SKI-1. Thus, we propose that the aforementioned site is the most likely internal autoprocessing site, with the qualification that PQ**RK**VF^{133}↓**R**SL**K**YAESD (SEQ. ID. NO. 45) may occur to a lesser extent (see Results and Fig. 22).--

Please replace the paragraph beginning at page 44, line 18, with:

--Other information regarding the substrate preferences of SKI-1 was obtained by replacing the P3' and P4' Ile and Pro residues of the C-terminal cleavage site peptide (III) by Leu and Glu (peptides IV and V) to create a very well processed SKI-1 substrate. While it would appear that the presence of an acidic residue at P4' significantly enhances the rate of substrate hydrolysis, it is also possible that the presence of Pro at P4' hinders efficient substrate processing. The presence of similar acidic residues at the P3' or P4' position of the two confirmed substrates of SKI-1 (peptides I and II) as well as in the prosegment internal cleavage site RSLK^{137}↓YAES (SEQ. ID. NO. 105) (Table II-A) lends support to the first argument. In addition to these residues, others also appear to play a role in SKI-1 substrate cleavage catalysis. The peptide pairs IV/V and X/XI both point to influences of positions N-terminal to the P4 residue. Interestingly, the efficiency of the truncated C-terminal peptide V is lower than that of peptide IV, whereas that of

the truncated internal (quenched) peptide XI is higher. Taken together, these data indicate the importance of aa at both the P and P' positions in SKI-1-mediated substrate hydrolysis.--

Please replace the paragraph bridging at pages 44 and 45, which starts with "The date presented in Fig. 24 [...]" with :

--The data presented in Fig. 24 indicate that SKI-1 functions most efficiently near neutral pH and at 2-3 mM Ca^{2+}. This is in general agreement with the conditions that reportedly prevail in the ER (28,29). However, closer examination of the data reveal that the pH optimum of SREBP-2 cleavage (peptide II, Fig. 24A) is actually 6.5, an observation that we confirmed using our purified SKI-1 preparation (*not shown*). This suggests that the processing of SREBP might occur outside of the ER, perhaps in the Golgi where pH values of ~6.5 have recently been reported (30,31). Indeed, there is now cellular evidence suggesting that SREBP cleavage may occur in the Golgi rather than in the ER (32,33). The pH optimum of SKI-1 appears to be dependent on the substrate employed; proBDNF (10) and its related peptide (I), appear to be well cleaved even at pH 5.5, suggesting that it could cleavethis (and possibly other substrates) in acidic endosome-like compartments where it was previously localized (10). On the other hand, cleavage of the internal, autocatalytic, prosegment processing site PQ**RK**VF**R**SL**K**^{137}↓YAESD (SEQ. ID. NO. 45) (Fig. 22B) is optimal at pH 8 (*not shown*), implying that this event, as we concluded from our biosynthesis assays, takes place most effectively in the ER. Overall, the pH and Ca^{2+} profiles of SKI-1 resemble those of the constitutively secreted PCs (1,13). The inhibitor profile of SKI-1 (10, Table III), showing that enzymatic activity is significantly inhibited by EDTA, EGTA and only high concentrations of *o*-phenanthroline, tend to discount the likelihood that SKI-1 is a transition metal-dependent proteinase. In fact, SKI-1 activity is inhibited by low concentrations of certain transition metals, such as Cu^{2+} and Zn^{2+}.--

Please replace the paragraph bridging at pages 45 and 46, which starts with "Directed by the observation [...]" with :

--Directed by the observation that peptides containing the primary processing site of the prosegment of PC1 are potent inhibitors of its activity, and that the C-terminal basic residues of furin and PC7 are essential for enzyme inhibition (34,35), we assessed the inhibitory potency of three SKI-1 recombinant propeptides. All of these end at sequences near the RRLL^{186}RA (SEQ. ID. NO. 106) cleavage site. Interestingly, the three prosegments displayed comparable inhibitory

potencies (Table V). Compared to proPC1 (34), pro-furin and proPC7 (35), the $K_{i(app)}$ values (Table V) are up to 250 fold higher. This suggests that the prosegment of SKI-1, although potentially inhibitory *in vivo*, may function more as a chaperone, catalyzing the productive folding of SKI-1. Indeed, since SKI-1 may be active in the ER (10,11), whereas the PCs are not (13,26), the lower inhibitory potency of the prosegment of SKI-1 may be adapted to the conditions prevailing in this cellular compartment. In the case of PCs, highly effective inhibition by the prosegment may be needed in order to ensure that these enzymes are activated only when they reach the TGN or secretory granules (1-3). The 14 kDa fragment, which represents the major secreted form of the prosegment, is tightly associated with SKI-1 (Fig. 19C) yet it is not inhibitory (*not shown*). Accordingly, this segment may serve a chaperonin-like function similar to that reported for the N-terminal 150 aa of 7B2 towards proPC2 (36,37).--

Please replace the paragraph beginning at page 46, line 16 with :

--In conclusion, the present work firmly establishes that SKI-1 is a Ca^{2+}-dependent subtilase with a reasonably neutral pH optimum, depending on the substrate employed. [ ]We also demonstrate that SKI-1 can cleave substrates C-terminal to Thr, Leu and Lys residues, thus providing direct, *in vitro* evidence that it is a candidate converting enzyme responsible for the generation of 28 kDa proBDNF (10) and SREBP-2 processing at site 1 (11). For efficient cleavage, it appears that substrates should contain a basic residue at P4 and an aliphatic one at P2 (Table II-A). Furthermore, aa at the P3' and P4' positions seem to exert an important discriminatory effect. The best substrate tested so far is the quenched flurorogenic substrate Abz-RSLK _ YAESDY(NO_2) (SEQ. ID. NO. 107), thereby providing a convenient and sensitive assay for SKI-1 activity. The present data demonstrate that only the full length SKI-1 prosegment is inhibitory. Thus, overexpression of this prosegment in cell lines may provide a novel method for inhibiting the cellular activity of this enzyme in a fashion similar to the that of over-expressed profurin and proPC7 (35). Finally, it is anticipated that precursor substrates other than the sterol regulating SREBPs (8) and the neurotrophin proBDNF (10) will be identified, thereby extending the spectrum of activity of this unique and versatile enzyme.--

Please replace Table II-A at page 47 with :

Peptide	P16	P12	P8	P4	P1	P4'	P8'	
I			K A G S R G L T	_	S L A D T F			(SEQ ID NO : 37)
II	G G A H D S D Q H P H	S G S G R S V L	_	S F E S G S G G				(SEQ ID NO : 38)
III		W H A T G R H S S R R L L	I	R A I P R				(SEQ ID NO : 39)
IV		W H A T G R H S S R R L L	_	R A L E				(SEQ ID NO : 40)
V			S R R L L	_	R A L E			(SEQ ID NO : 41)
VI [1]		W Q S S R P L R R A S L	-	S L G S G				(SEQ ID NO : 42)
VII [1]			R A I P R Q V A	-	Q T L Q A D V			(SEQ ID NO : 43)
VIII [2]			P Q R K V F	-	R S L			(SEQ ID NO : 44)
IX [2,3]		P Q R K V F R S L K	_	Y A E S D				(SEQ ID NO : 45)
X		Abz-V F R S L K	_	Y A E S D	Y(NO_2)	-A		(SEQ ID NO : 46)
XI		Abz-R S L K	_	Y A E S D	Y(NO_2)	-A		(SEQ ID NO : 47)

Please replace Table II-1 at page 51 with:

TABLE VI
PRECURSOR CLASSIFICATION BASED ON HYDROPHOBIC AND/OR SMALL AMINO ACID CLEAVAGE

Precursor protein	Cleavage site sequence			
	P8 P7 P6 P5 P4 P3 P2 P1		**P1' P2' P3' P4' P5' P6' P7' P8'**	
(h)proBDNF	**Lys**-Ala-Gly–Ser–**Arg**–Gly–**Leu-Thr**	↓	Ser-Leu-Ala-Asp-Thr-Phe-Glu-His	(SEQ ID NO: 48)
(r)proBDNF	**Lys**-Ala-Gly–Ser–**Arg**–Gly–**Leu-Thr**	↓	Thr-Thr-Ser-Leu-Ala-Asp-Thr-Phe	(SEQ ID NO: 49)
(h)proSKI-1	**Arg**-His-Ser–Ser–**Arg**–Arg–**Leu-Leu**	↓	Arg–Ala-Ile-Pro-Arg-Gln-Val-Ala	(SEQ ID NO: 50)
	Arg-Lys–Val–Phe–**Arg**–Ser-**Leu-Lys**	↓	Tyr-Ala-Glu-Ser-Asp-Pro-Thr-Val	(SEQ ID NO: 51)
	Thr-Pro-Gln-Arg-**Lys**–Val–**Phe–Arg**	↓	Ser-Leu-Lys–Tyr-Ala-Glu-Ser-Asp	(SEQ ID NO: 52)
	Val-Thr-Pro-Gln-**Arg**-Lys–**Val–Phe**	↓	Arg–Ser-Leu-Lys-Lys-Tyr-Ala-Glu	(SEQ ID NO: 53)
(h)SREBP-2	Ser-Gly-Ser-Gly-**Arg**-Ser-**Val-Leu**	↓	Ser-Phe-Glu-Ser-Gly-Ser-Gly-Gly	(SEQ ID NO: 54)
(h)SREBP-1a	**His**-Ser-Pro-Gly-**Arg**-Asn-**Val-Leu**	↓	Gly-Thr-Glu-Ser-Arg-Asp-Gly-Pro	(SEQ ID NO: 55)
(r)pro-Relaxin (B-chain)	Ala-Ser-Val-Gly-**Arg**-Leu-**Ala-Leu**	↓	Ser-Gln-Glu-Glu-Pro-Ala-Pro-Leu	(SEQ ID NO: 56)
(h)pro-CCK (CCK5)	**Arg**-Ile-Ser-Asp-**Arg**-Asp-**Tyr-Met**	↓	Gly-Trp-Met-Asp-Phe-Gly-Arg-Arg	(SEQ ID NO: 57)
(r)pro-Somatostatin (Antrin)	Asp-Pro-**Arg**-Leu-**Arg**-Gln-**Phe-Leu**	↓	Gln-Lys-Ser-Leu-Ala-Ala-Ala-Thr	(SEQ ID NO: 58)
(b)Chromogranin A (82↓83)	Leu-Leu-**Lys**-Glu-Leu-Gln-Asp-**Leu**	↓	Ala-Leu-Gln-Gly-Ala-Lys-Glu-Arg	(SEQ ID NO: 59)
(b)Chromogranin A (309↓310)	Met-Ala-**Arg**-Ala-Pro-Gln-**Val-Leu**	↓	Phe-Arg-Gly-Gly-Lys-Ser-Gly-Glu	(SEQ ID NO: 60)
(b)Chromogranin B (629↓630)	Glu-Leu-Glu-Asn-Leu-Ala-Ala-**Met**	↓	Asp-Leu-Glu-Leu-Gln-Lys-Ile-Ala	(SEQ ID NO: 61)
(b)Chromogranin B (634↓635)	Ala-Ala-Met-Asp-Leu-Glu-**Leu-Gln**	↓	Lys-Ile-Ala-Glu-Lys-Phe-Ser-Gly	(SEQ ID NO: 62)
(r)pro-Renin	**Lys**-Ser-Ser-Phe-Thr-Asn-**Val-**	↓	Ser-Pro-Val-Val-Leu-Thr-Asn-Tyr	(SEQ ID NO: 63)
(r)α-Endorphin	**Lys**-Ser-Gln-Thr-Pro-Leu-**Val-Thr**	↓	Leu-Phe-Lys-Asn-Ala-Ile-Ile-Lys	(SEQ ID NO: 64)
(r)γ-Endorphin	Ser-Gln-Thr-Pro-Leu-Val-**Thr-Leu**	↓	Phe-Lys-Asn-Ala-Ile-Ile-Lys-Asn	(SEQ ID NO: 65)
(r)pro-AVP (CPP)	Gly-Pro-Ala-Arg-**Glu**-Leu-**Leu-Leu**	↓	Arg-Leu-Val-Gln-Leu-Ala-Gly-Thr	(SEQ ID NO: 66)
(h)ADAM-10 (kuzbanian)	Leu-Leu-**Arg**-Lys-**Lys**-Arg-Thr-**Thr**	↓	Ser-Ala-Glu-Lys-Asn-Thr-Cys-Gln	(SEQ ID NO: 67)
(h)β-APP				
β-Secretase site	Glu-Glu-Ile-Ser-**Glu**-Val-Lys-**Met**	↓	Asp-Ala-Glu-Phe-Arg-His-Asp-Ser	(SEQ ID NO: 68)
β-Secretase site (*Swedish*)	Glu-Glu-Ile-Ser-**Glu**-Val-**Asn-Leu**	↓	Asp-Ala-Glu-Phe-Arg-His-Asp-Ser	(SEQ ID NO: 69)
$\beta\varepsilon_1$-Secretase site	Ile-Ser-Glu-Val-**Lys**-Met-Asp-**Ala**	↓	Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr	(SEQ ID NO: 70)
$\beta\varepsilon_2$-Secretase site	Glu-Phe-**Arg-His-Asp-Ser-Gly-Tyr**	↓	**Glu-Val-His-His-Gln-Lys-Leu-Val**	(SEQ ID NO: 71)

Please replace the paragraph beginning at page 52, line 26 with :

--We have shown that expression of full length SKI-1 prosegment (22-24 kDa with sequence ending at the secondary cleavage sequence RHSSRRLL (SEQ. ID. NO. 89)) Inhibits SKI-1 activity in stable HK 293 cell lines (Example 2). However, since the prodomain of SKI-1 is processed at an internal primary cleavage site RKVFRSLK (SEQ. ID. NO. 94) to give a 14 kDa N-terminal fragment (Fig. 29A&B) we predict that mutation of this site will generate an even more effective SKI-1 inhibitor. In fact, in the case of the mouse PC5 prodomain we have shown that mutation of the internal prosegment cleavage site does in fact generate a inhibitor of integrin $h\alpha_4$ 150 kDa processing to 80kDa and 70kDa species (Fig. 15).--

Please replace EXAMPLE 6 beginning at page 53, line 1, with:

--EXAMPLE 6

SKI-1 Peptide Substrates for fluorescence resonance energy transfer (FRET) – Based Proteolysis Assays

A large number of synthetic peptides based on potential cleavage sites in the hSKI-1 prodomain, proBDNF and the loop region of SREBP-2 were synthesized.

These are:

(i) hSKI-1 (156-172)

Trp-Gln-Ser-Ser-Arg-Pro-Leu-Arg-Arg-Ala-Ser-Leu↓Ser-Leu-Gly-Ser-Gly (SEQ. ID. NO. 42)

(ii) hSKI-1 (174-191)

Trp-His-Ala-Thr-Gly-Arg-His-Ser-Ser-Arg-Arg-Leu-Leu↓Arg-Ala-Ile-Pro-Arg (SEQ. ID. NO. 39)

(iii) hSKI-1 (174-188+Leu+Glu)

Trp-His-Ala-Thr-Gly-Arg-His-Ser-Ser-Arg-Arg-Leu-Leu↓Arg-Ala-Leu-Glu (SEQ. ID. NO. 40)

(iv) hSKI-1 (181-188+Glu)

Ser-Ser-Arg-Arg-Leu-Leu↓Arg-Ala-Ile-Glu (SEQ. ID. NO. 72)

(v) hSKI-1 (187-201)

Arg-Ala-Ile-Pro-Arg-Gln-Val-Ala↓Gln-Thr-Leu-Gln-Ala-Asp-Val (SEQ. ID. NO. 43)

(vi) hSKI-1 (128-136)

Pro-Gln-Arg-Lys-Val-Phe-Arg-Ser-Leu ((SEQ. ID. NO. 44)

(vii) hSKI-1 (128-142)

Pro-Gln-Arg-Lys-Val-Phe-Arg-Ser-Leu-Lys↓Tyr-Ala-Glu-Ser-Asp ((SEQ. ID. NO. 45)

(viii) hProBDNF (50-63)

Lys-Ala-Gly-Ser-Arg-Gly-Leu-Thr↓Ser-Leu-Ala-Asp-Thr-Phe (SEQ. ID. NO. 37)

(ix) SREBP-2 27 mer

Gly-Gly-Ala-His-Asp-Ser-Asp-Gln-His-Pro-His-Ser-Gly-Ser-Gly-Arg-Ser-Val-Leu↓Ser-Phe-Glu-Ser-Gly-Ser-Gly-Gly (SEQ ID NO: 38)

(x) SREBP-2 10 mer

Ser-Gly-Ser-Gly-Arg-Ser-Val-Leu↓Ser-Phe-Glu-Ser (SEQ. ID. NO. 73).--

Please replace the paragraph beginning at page 54, line 10, with:

--(a) QSKI (132-142):

Abz-Val-Phe-Arg-Ser-Leu-Lys ↓Tyr-Ala-Glu-Ser-Asp-Tyr(NO_2)-Ala (SEQ. ID. NO. 46)

(b) QSKI (134-142):

Abz-Arg-Ser-Leu-Lys ↓ Tyr-Ala-Glu-Ser-Asp-Tyr(NO_2)-Ala (SEQ. ID. NO. 47)

(c) QSKI (178-188)

Abz-Arg-His-Ser-Ser-Arg-Arg-Leu-Leu ↓ Arg-Ala-Ile-Tyr(NO_2)-Ala (SEQ. ID. NO.74)

(d) QSKI (181-187+Leu+Glu)

Abz-Ser-Arg-Arg-Leu-Leu ↓ Arg-Ala-Leu-Glu-Tyr(NO_2)-Ala (SEQ. ID. NO. 75)

(e) QBDNF (47-58)

Abz-Asn-Gly-Pro-Lys-Ala-Gly-Ser-Arg-Gly-Leu-Thr ↓ Ser-Tyr(NO_2)-Ala (SEQ. ID. NO. 76)--